of the corresponding DNA sequence" and "recognises residual single-stranded" (claim 17); "capable of recognizing analyte in the liquid sample", "capable of recognizing and binding with either another part of the analyte or the residual probe", "recognises", and "insignificant effect" (claims 19-25, 27, 29-31); "capable of binding" (claims 22-25); "capable of binding to" (claim 31).

Claims 1 and 19 have been further rejected under 35 U.S.C. §112, second paragraph, as being incomplete for allegedly omitting essential steps, such omission amounting to a gap between the steps. In particular, the Examiner alleges that claims 1 and 19 both fail to recite a step that states that the object of each method as recited in the preamble of the respective claims is accomplished.

Claim 26 has been further rejected under 35 U.S.C. §112, fourth paragraph, as being of improper dependent form for failing to further limit the subject matter of a previous claim because claim 26 allegedly adds no further limitations to the process of claim 11.

The Examiner also contends that claims 1, 7, 8, 12, 17-21 and 27 are anticipated by the disclosure of the Cheung patent (U.S. Patent No. 5,132,242).

Claims 1-6, 7, 11-31 are further alleged to be unpatentable under 35 U.S.C. §103 in view of the combined disclosures of the Ekins patent (U.S. Patent No. 5,171,695) in view of the aforesaid Cheung patent. Based on the disclosures of the cited references, the Examiner maintains that it would have been prima facie obvious to one of ordinary skill in the art to use fluorescent microspheres as a label in the method of the Ekins patent to increase the sensitivity and resolution of substrates which heretofore were not detectable, such as precise resolution of antigenic sites and rapid high resolution mapping of single gene sequences, as allegedly suggested by the Cheung patent.

A separate ground of rejection under §103 is raised with respect to claims 8 and 9, based on the combined disclosures of the aforesaid Ekins patent, in view of the

aforesaid Cheung patent and further in view of the Mandle et al. patent (U.S. Patent No. 4,372,745). In support of this ground of rejection, the Examiner contends that it would have been prima facie obvious to one of ordinary skill in the art to perform the method of Ekins using fluorescent labeled microspheres of Mandle et al. as a variant of the microspheres described by Cheung to provide a novel class of conjugated microencapsulated fluorescer/biological compositions useful in the detection of various biological analytes of interest.

Claim 10 is also alleged to be unpatentable under 35 U.S.C. §103 based on the combined disclosures of the aforesaid Ekins patent, in view of the aforesaid Cheung patent, and further in view of the aforesaid Mandle et al. patent and the Wagner et al. patent (U.S. Patent 4,978,625). Based on the combined disclosure of the four (4) cited references, the Examiner maintains that it would be prima facie obvious to perform the method of Ekins using fluorescent microspheres, using oil soluble fluorescent dyes, to provide dyes which will not leak out of a microsphere so as to be able to detect a fluorescent signal without rupturing the microsphere.

In addition to the above-noted rejections, the Examiner has required submission of an Abstract in accordance with 37 C.F.R. §1.72(b).

The Examiner has also made certain additional prior art references of record, which are deemed by the Examiner to be pertinent to applicants' disclosure. Specifically, U.S. Patent No. 5,028,545 to Soini and U.S. Patent No. 4,732,847 to Stuart et al. have been made of record, but no rejection has been based on the last-mentioned two (2) references.

In accordance with present amendments, an "Abstract of the Disclosure", in conformity with the requirements of 37 C.F.R. §1.72(b), is submitted herewith.

Claims 1 to 31 have been cancelled and claims 32 to 47 have been added to more particularly point out the subject matter of the invention. These new claims omit certain of the claim terminology found objectionable by the Examiner. However, a few of the challenged expressions have been

retained in the new claims.

New claim 32 corresponds generally to original claim 1 and clarifies the key features of the invention including the use of the capture binding agent immobilised at high density on a solid support in the form of microspots and the use of developing binding material labelled using microspheres. New claim 33 generally corresponds to original claim 2 and relates to the preferred feature that a small amount of capture binding agent is used. New claim 34 corresponds to original claim 3 and also relates to the small amount of capture binding agent required. Support for claim 34 is provided at page 11, lines 1-2. New claim 35 generally corresponds to original claim 3 and relates to a preferred range of capture binding agent densities. New claim 36 corresponds to original claim 5 and specifies a preferred upper limit on the area of the microspots.

New claim 37 corresponds generally to original claim 29 and relates to measurement of a plurality of analyte concentrations using a support having a plurality of microspots containing different capture binding agents specific for each of the analytes. New claim 38 also corresponds generally to original claim 29 and relates to the possibility of using a universal label to make the developing binding material. New claim 39 corresponds generally to original claim 31 and specifies a preferred way of linking the microspheres containing the universal label to the developing binding material(s). New claim 40 corresponds generally to original claim 9 and specifies that the label is contained within the microspheres instead of being attached to the surface of the microspheres. New claim 41 corresponds generally to original claim 9 and relates to the preferred use of fluorescent labels. New claim 42 corresponds generally to original claim 14 and specifies a preferred step used to ameliorate clumping between the microspheres by blocking them. New claim 43 corresponds generally to original claim 16 and specifies that the capture binding agent and the developing binding materials are antibodies. New claim 44 corresponds

generally to original claim 17 and specifies the use of oligonucleotides when nucleic acid analytes are being used. New claim 45 corresponds generally to original claim 19 and relates to a method of detecting nucleic acid sequences using an analogous method to claim 32. New claims 46 and 47 correspond generally to original claims 22 and 25, respectively, and relate to a kit providing elements carrying out the assay process of claims 32 to 45.

The reference to a comparison with a dose response curve recited in original claim 1 was omitted from new claim 32. Use of a dose response curves in assays of this type are conventional and it is settled law that claims need not recite factors that are within the level or ordinary skill in the art. In re Skrivan, 166 U.S.P.Q. 85 (C.C.P.A. 1970); accord Ex parte Rinehart, 10 U.S.P.Q.2d 1719 (P.T.O. B.P.A.I. 1989) (no reason to require process limitations which are within the ordinary skill of the worker in the relevant art).

In addition, the step described by the phrase "thereby allowing the presence of said one or more target nucleic acid sequences to be detected" was added to new claim 45 to supply the detection step recited in the preamble to that claim.

No new matter has been introduced into this application by reason of the amendments presented herewith.

Before addressing the specific grounds of rejection, a brief review of the applicant's invention may be helpful to focus on those aspects that are believed to distinguish over the prior art. The present invention concerns an assay process and kit which uses binding agent specific for one or more analytes which is immobilized on a solid support at high density as one or more microspots, in combination with a developing binding material labelled with microspheres. A key unexpected advantage of this methodology is that the sensitivity of the assay is dramatically improved, leading to sensitivities in the detection and determination of the concentration of analytes well beyond those which could be determined using conventional techniques.

The assay sensitivity is demonstrated in examples 7 and 10 of the instant application which describe TSH assays having a sensitivities of 0.0002mU/litre. To put this in context, the applicant refers to an article by I. D. Hay et al. relating to measuring the concentration of the same analyte which appeared in Clinical Chemistry, Vol. 37, No. 11 (1991), pp 2002-2008. In this article, the American Thyroid Association states on page 2007, right hand column, last paragraph, that existing assays:

> cannot readily differentiate between mildly subnormal values (0.01-0.1 milli-int. unit/L) seen in hospitalized or T4-treated patients and profoundly low values (<0.01 milli-int. unit/L) typical of thyrotoxicosis.

unexpected result

Thus, the assay exemplified in the instant application can achieve sensitivities at least **50-500 times better** than the sensitivity levels that the American Thyroid Association describe as being poorly differentiated by best assays available in the prior art, just before the priority date of the application. Practically, the present invention improves the sensitivity of the determination of TSH (and other analytes). In the case of TSH, the assay of the invention improves the data made available to the physician and so the treatment made available to the patient.

Applicants believe that the use of high surface densities of capture binding agent immobilized in very small areas improves the signal-to-noise ratio when the signal from the label is measured, as the high density of capture binding agent helps to reduce the non-specific binding of the developing binding material, as compared to conventional approaches which tend to use large amounts of capture binding agent spread evenly over relatively larger surface areas. The high surface density may also help to improve the strength of the binding between the analyte and the capture binding agent or developing binding material, as a single analyte molecule can be bound by multiple binding material molecules, greatly enhancing the strength of this interaction relative to the non-specifically bound material. This is because in multiple

binding reactions, the strength of the binding increases by a factor of the product of the individual affinity constants. The net result of using the claimed assay is a significant increase in sensitivity.

As a result of the foregoing amendments, any indefiniteness that may have existed in claims 1 to 31 by reason of the use of the expressions "a small amount", "insignificant effect on the concentration of the analyte", "provided on their surface with negatively charged or positively charged groups", "colour range compatible with a standard filter set", "signal strength capable of being determined", "twin-stranded DNA", "recognises another part of the corresponding DNA sequence", "recognises residual single-stranded", "capable of recognizing analyte in the liquid sample", "capable of recognizing and binding with either another part of the analyte or the residual probe", "recognises", "insignificant effect", "capable of binding", "capable of binding to" has been eliminated.

Furthermore, any indefiniteness that may have existed in original claim 1 has been eliminated in new claim 32 by the omission of the reference to a dose response curve. Any indefiniteness that may have existed in original claim 19 has been eliminated in new claim 45 by the addition of the phrase "thereby allowing the presence of said one or more target nucleic acid sequences to be detected."

The rejection of original claim 26 under 35 U.S.C. §112, fourth paragraph, is rendered moot in view of the cancellation of claim 26.

Thus the only matters remaining to be addressed are the 35 U.S.C. §112 rejections based on the claim terminology "high density", "one or more microspots" and "capable of hybridizing" and the prior art rejections under 35 U.S.C. §§102(e) and 103. These remaining grounds of rejection are respectfully traversed.

1. Claims 32 to 47 Clearly Satisfy the Requirements of 35 U.S.C. §112, Second Paragraph

The relevant inquiry in determining whether a given claim satisfies the requirements of 35 U.S.C. §112, second paragraph, is whether the claim sets out and circumscribes a particular area with a sufficient degree of precision and particularity, such that the metes and bounds of the claimed invention are reasonably clear. In re Moore, 169 U.S.P.Q. 236 (C.C.P.A. 1971). Applicant respectfully submits that with respect to new claims 32 to 47 of this application, such inquiry must be answered in the affirmative.

The definiteness of claim language may not be analyzed in the abstract, but must be considered in light of the teachings of the prior art and of the particular application disclosure, as it would be interpreted by one having ordinary skill in the art. In re Moore, supra.

The term "high density" is retained in the independent claims because a person of ordinary skill in the art would know what constitutes a conventional density as well as what constitutes a comparatively high density for a particular binding agent. Furthermore, the method of the invention is applicable to a wide range of different capture binding agents and a high density of those different agents would vary depending on factors including the steric size of a particular capture binding agent.

An upper range of microspots has not been specified because applicants submit that this is not a cause of indefiniteness and such a limit would place an undue restriction on the claims. The assay of the invention can perform hundreds or thousands of assays simultaneously and the number of microspots does not change the fundamental nature of the invention.

The hybridizing language was adopted in new claims 44 and 45. However, applicants submit that language specifying that the nucleic acid and oligonucleotides are "capable of hybridizing" to each other is in itself clear and

does not require the introduction of the specific conditions under which hybridization takes place.

In summary, Applicant's position with respect to the rejection of Claims 1 to 31 based on 35 U.S.C. §112, second paragraph, is that any person skilled in the art having Applicant's disclosure and claims before him or her would be apprised, to a reasonable degree of certainty, as to the exact subject matter encompassed within the claims. Nothing more is required under 35 U.S.C. §112, second paragraph. Accordingly, the 35 U.S.C. §112, second paragraph rejection of Claims 1 to 31 is untenable and should be withdrawn. Furthermore, one skilled in the art reading applicants' pending claims 32 to 47 in light of the present specification would clearly not be confused as to what those claims, considered as a whole, would preclude others from doing in future enterprise.

2. The Subject Matter of Claims 32 to 47 is Patentably Distinguishable over the Disclosure of Cheung

The 35 U.S.C. §102(e) rejection based on the Cheung patent is inapplicable to new claims 32 to 47 and should be withdrawn.

Rejections under 35 U.S.C. §102(e) are proper only when the claimed subject matter is identically disclosed or described in the prior art. In re Arkley, 172 U.S.P.Q. 524 (C.C.P.A. 1972). Applying this rule of law to the present case, the lack of novelty rejection based on the Cheung patent cannot be maintained because the Cheung patent plainly fails to identically disclose or describe the subject matter of the rejected claims. There is no disclosure in the Cheung patent of an assay that overcomes non-specific binding and enhances sensitivity, having the features set forth in the applicants' new claims. Furthermore, the experimental results reported in the Cheung patent examples are solely concerned with visualizing cell surface antigens or chromosomal DNA, e.g., detecting the presence of relatively large concentrations of analytes, rather than determining the concentration of

analytes or the presence of analytes at very low concentrations as set forth in applicant's new claims 32 to 47. Accordingly, the 35 U.S.C. 102(e) rejection of these claims based on the Cheung article is untenable and should be withdrawn.

3. The Subject Matter of Claims 32 to 47 is Patentably Distinguishable Over the Combined Disclosure of Ekins, Cheung, Mandle et al. and Wagner et al.

The 35 U.S.C. §103 rejections based on the disclosures of the Cheung patent and the Ekins patent, and further considered in view of the Mandle patent, with or without reference to the Wagner et al. patent is inapplicable to new claims 32 to 47 and should be withdrawn.

As noted by the PTO Board of Appeals in Ex parte Wolters, 214 U.S.P.Q. 735 (P.T.O. B.P.A.I. 1979), the burden of establishing a prima facie case of obviousness falls upon the Examiner. In determining whether a case of prima facie obviousness exists, it is necessary to ascertain whether or not the disclosures of the cited prior art would appear to be sufficient to one of ordinary skill in the art to make the claimed substitution, combination or other modification. In re Lalu, 223 U.S.P.Q. 1257 (Fed. Cir. 1984). Merely because it is possible to find two prior art disclosures which might be combined in such a way as to arrive at the claimed subject matter does not make the combination of disclosures obvious unless the art also contains something to suggest the desirability of the proposed combination. In re Imperato, 179 U.S.P.Q. 730 (C.C.P.A. 1973).

In the present case, there is nothing to suggest the desirability of combining the disclosures of the cited references in the manner proposed by the Examiner. The Ekins patent discloses use of two labels and a small amount of binding agent to provide an assay which is independent of the volume of the sample and the absolute amount of binding agent.

However, Ekins does not disclose both the use of microspots containing high densities of binding agent and the use of microspheres as labels as required in the applicants' new claims. The Cheung patent discloses control and manipulation of the surface chemistry of very small latex microspheres to increase the number of fluorescent molecules attached to their surface of the spheres. However, Cheung does not disclose ways to overcome non-specific binding or to enhance sensitivity which were known drawbacks in the application of labelled microspheres to assays. Cheung also does not disclose a method for determining the concentration of analytes or the presence of analytes at very low concentrations. Thus, neither of the above cited references contain a suggestion to combine the disclosures to arrive at the assay of the invention.

The Examiner also contends that based on the disclosures of Ekins and Cheung in combination with Mandle et al. it would have been prima facie obvious to perform the method of Ekins using fluorescent labeled microspheres of Mandle as a variant of Cheung to provide conjugated microencapsulated fluorescer/biological compositions to detect analytes of interest. Mandle discloses the use of microspheres containing a large amount of fluorescent dye which binds to the analyte to form a complex which is then separated from the test sample and the microspheres are disrupted to release the dye into the solution. However, Mandle does not disclose improvement in sensitivity of microspot assays or a method for analysis of multiple analytes. Thus Mandle et al. also does not suggest combining the cited references to obtain the assay of the invention.

The Examiner further contends that based on the disclosures of Ekins and Cheung in combination with Mandle et al. and Wagner et al., it would have been prima facie obvious to perform the method of Ekins using fluorescent microspheres containing oil soluble fluorescent dyes to provide dyes which will not leak out of a microsphere. Wagner et al. discloses the use of water insoluble dyes entrapped in the lipid portion

of a liposome for performing immunoassays. Wagner does not disclose the use of microspheres or microspots in performing immunoassays. Therefore, the cited Ekins, Cheung, Mandle, and Wagner references do not suggest that the combined disclosures would result in the assay of the invention.

Evidence demonstrating the unexpected and surprising sensitivity in detecting and quantitatively analyzing small concentrations of analytes in accordance with the present invention is set forth in Examples 6 and 7 at pages 26-27 and Example 10 at pages 28-29 of the present specification. Example 6 describes an assay for thyroid stimulating hormone (TSH) which was carried out using two monoclonal antibodies directed at different epitopes on the TSH molecule as capture and developing antibodies. The TSH samples were incubated overnight in the presence of capture antibodies deposited as microspots in microtitre wells and developing antibody covalently coupled to carboxylate-modified latex FluoSpheres. The sensitivity of the assay (based on measurements of the standard deviation of the zero dose estimate) was 0.002 mU/litre. In Example 7, even greater sensitivity was obtained when the total incubation time was reduced to one hour and the size of microspheres was increased to 0.12 μM diameter. The sensitivity of the assay was thereby increased ten-fold to 0.0002 mU/litre, based on measurements of the standard deviation of the zero dose estimate. Example 10 describes a dual-label assay in which the capture antibody was labelled. The sensitivity of the assay in Example 10 was also 0.0002 mU/litre, based on measurements of the standard deviation of the zero dose estimate. The above examples show the unexpectedly high sensitivities obtained using the assay of the invention and rebut the Examiner's rejection based on prima facie obviousness.

The evidence of unexpected results presented in applicants' specification must be considered in reaching a determination as to whether the claimed invention as a whole would have been obvious under 35 U.S.C. §103. In re Margolis, 228 U.S.P.Q. 940 (Fed. Cir. 1986). The Examiner has not given

any indication that this evidence has been duly considered.

For all of the reasons set forth above, the conclusion is inescapable that those of ordinary skill in the art would not have found it obvious to combine the cited references so as to arrive at the binding assay claimed by applicant herein, which involves determination of the concentration or presence of small quantities of analyte. Thus the rejection of original claims 1 to 31 under 35 U.S.C. §103 based on the combination of Cheung, Ekins, Mandle et al. and Wagner et al., considered singly or in any combination, is untenable and should not be applied to new claims 32 to 47.

Finally, as further indication of the patentability of the present invention, the Examiner's attention is respectfully directed to the International Preliminary Examination Report (copy attached) issued in the underlying PCT application, in which the assay of the present invention was determined to satisfy all of the patentability criteria of the PCT. Moreover, the applicants' assay was presumably found to be patentably distinguishable over WO 88/01058 which corresponds to the Ekins '695 patent and which was cited in the International Search Report in the underlying PCT application. The aforementioned International Preliminary Examination Report includes the following statement regarding the patentability of the assay of the invention:

> Claims 1-25 meet the requirements of both novelty and inventive step because there is not disclosure nor suggestion in the documents cited in the International Search Report of a binding assay or kit which employs microspheres and capture binding agent which is immobilized at high density to a solid support. Moreover, the advantages displayed by such an assay or kit could not have been predicted from an assay or kit which combined the use of microspheres and capture binding agent immobilized at high density to a solid support which features have been disclosed separately in the prior art.

This reasoning clearly refutes the Examiner's obviousness rejection in the present application.

In addition, the WO 88/01058 reference was categorized as a category A document indicating that it is not particularly relevant to the assay of the invention.

As noted previously, several references (U.S. Patent No. 5,028,545 to Soini and U.S. Patent No. 4,732,847 to Stuart et al.) were listed in the June 20, 1995 Official Action, but were not applied against any of the claims. That being the case, no detailed discussion of these references would appear to be in order. Suffice it to say that the last-mentioned references fail to provide evidence of lack of novelty or obviousness with respect to the subject matter of claims 32 to 47.

The Examiner is correct in presuming (at the bottom of page 9 of the June 20, 1995 Office Action) that the subject matter of the various claims was commonly owned at the time the inventions covered thereby were made.

It is respectfully requested that the request for new drawings set forth in the June 20, 1995 Office Action be held in abeyance, pending the indication of allowable subject matter.

In view of the foregoing remarks, it is respectfully urged that the rejections set forth in the June 20, 1995 Official Action be withdrawn and that this application be passed to issue and such action is earnestly solicited.

Respectfully submitted,
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Enclosures: Exhibit A - Clinical Chemistry, Vol. 37, No. 11 (1991), pp 2002-2008
Exhibit B - International Preliminary Examination Report from PCT/GB92/01892